

Use of attenuated sporozoites in the immunization of human volunteers against falciparum malaria*

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Three human volunteers were successfully protected against sporozoite challenge by immunization with attenuated sporozoites of the Tamenie strain of Plasmodium falciparum from Ethiopia. The immunizing sporozoites were attenuated by exposing infected Anopheles stephensi mosquitos to X-rays at a dose of at least 120 Gy (12 000 rad). These irradiated, infected mosquitos were allowed to feed upon volunteers, thereby inoculating sporozoites into their blood stream. During the 10- to 38-week period of immunization, volunteers were exposed 6-8 times, at irregular intervals, to a total of 440-987 irradiated infected mosquitos. Protection against challenge by nonirradiated infected mosquitos lasted for at least 8 weeks, but not 16 weeks, after the last immunization with irradiated sporozoites. By contrast, volunteers who were exposed to a total of 200 or fewer irradiated infected mosquitos on 2-4 occasions were not protected upon challenge. Immunization by a sufficient number of irradiated mosquitos infected with the chloroquine-sensitive Tamenie strain from Ethiopia also protected against challenge with the chloroquine-resistant Marks strain of P. falciparum from Viet Nam. The results obtained in these studies suggest that immunization with attenuated sporozoites may be a useful method of protecting small groups of nonimmune individuals living in endemic areas. These findings should encourage further efforts to develop a sporozoite vaccine against human malaria.

In view of the encouraging results obtained by immunizing mice with irradiated sporozoites of *Plasmodium berghei* (1), a collaborative study was initiated in 1971 between investigators at Rush-Presbyterian-St Luke's Medical Center, Chicago, Illinois and the Naval Medical Research Institute (NMRI), Bethesda, Maryland, to determine whether protection could also be induced in human volunteers by exposing them to irradiated sporozoites of *Plasmodium falciparum*. As it was deemed ethically unacceptable to inoculate crude suspensions of sporozoites into volunteers, exposure to sporozoites was achieved by

allowing participants in these studies to be bitten by irradiated infected mosquitos. It was realized, of course, that only a small proportion of sporozoites in the salivary glands would be released by mosquitos, that sporozoite inocula would probably be considerably lower than those used in previous animal experiments, and that the quantity of immunizing antigen would not be known. On the other hand, the results of animal experiments were so promising that an attempt was made to duplicate these results with *P. falciparum* in man.

The studies were carried out at the Stateville Correctional Center, Joliet, Illinois. Since human beings had never before received irradiated sporozoites, 5 investigators at NMRI, including 2 of the authors of this paper, volunteered to be exposed to irradiated infected mosquitos in order to establish the safety of this procedure. They were bitten by several hundred irradiated infected or noninfected mosquitos once a week for a period of 6 weeks. Thorough clinical and laboratory examinations over a period of several months failed to show any evidence of adverse side-effects to the sporozoites, although local reactions to the mosquito bites were observed in the case of 1 volunteer. After completion of these preliminary studies, investigations involving inmate volunteers were initiated to ascertain whether irradiated sporozoites could confer protection against human falciparum malaria.

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parum malaria. The results of these studies, obtained between 1972 and 1975, are described in this report.

MATERIALS AND METHODS

These studies involved the participation of 136 inmates of the Stateville Correctional Center who had volunteered for the programme. Volunteers were healthy men between 21 and 40 years of age who had no previous exposure to malaria. Detailed information about the study and its potential risks was given to each individual and the voluntary nature of participation, including the right to withdraw at any time, was emphasized. The studies were approved by the Human Investigation Committee of Rush-Presbyterian-St Luke's Medical Center and they were conducted according to ethical and medical procedures established previously (2).

In these studies, 84 volunteers participated as gametocyte donors. They were usually infected with the Tamenie strain (Ethiopia) of chloroquine-sensitive *P. falciparum*. In some instances, the Marks strain (Viet Nam) of chloroquine-resistant *P. falciparum* or the Chesson strain of *P. vivax* was used. Infections were induced through the bites of infective mosquitoes or by intravenous inoculation of small aliquots of parasitized blood. Thick blood films were prepared at least once a day, stained with Giemsa stain, and examined by the method of Earle & Perez (3) for asexual forms and for gametocytes. Participants were admitted to the malaria ward on the same day that they developed patent parasitaemia or clinical symptoms and they were kept under close medical supervision throughout the entire duration of the study. Early in the clinical course of malaria, gametocyte donors received subcurative doses of chloroquine and quinine to relieve symptoms and to prevent the occurrence of high levels of parasitaemia. Although suppression of asexual parasites was essential for the safety and comfort of participants, it often prevented the subsequent development of an adequate number of gametocytes for infecting mosquitoes. Consequently, only about one-third of the men developed a gametocytaemia capable of inducing heavy sporozoite infections.

The mosquitoes used for transmission of sporozoites were *Anopheles stephensi* reared at the Stateville insectary. After gametocyte donors had developed a parasite count of at least 100 gametocytes per mm³ of blood, mosquitoes were allowed to bite them. Engorged mosquitoes were then returned to the insectary in a holding cage. Six days later, 5 mosquitoes from the cage were examined for the presence of oocysts. On each of days 10, 12, 14, and 16, the salivary glands of 5 additional mosquitoes were examined for the pres-

ence of sporozoites. Batches of mosquitoes that were heavily infected (at least 25% of mosquitoes with more than 1000 sporozoites per mosquito) were irradiated between 12 and 18 days after their blood meal.

Irradiation of infected and noninfected mosquitoes was performed at the Radiation Physics Section of Rush-Presbyterian-St Luke's Medical Center by means of a Westinghouse 220 kV (peak) ortho voltage X-ray machine. The tube was operated at a peak voltage of 220 kV and 18 mA and the quality of the beam was defined by use of a 0.5-mm copper filter. The distance between the surface of the sample and the target was 10.0 cm and the exposure rate was 9.1×10^{-3} C/(kg-s) (2100 R/min). The exposure time was determined by measurement with a Victoreen R meter prior to the exposure of the sample and, in addition, a lithium fluoride disc was used to confirm the minimum dosage of 120 Gy (12 000 rad) for each sample irradiated. Mosquitoes were irradiated in a plastic container measuring 1.0 cm in height and 9.4 cm in diameter. Back-scatter material was provided by placing the container on 13.0 cm of water. After X-irradiation delivering a minimum of 120 Gy, mosquitoes were transported back to the malaria ward and, within approximately 60 min after irradiation, they were allowed to bite the appropriate participants in these studies.

The actual immunization studies involved the participation of 52 volunteers. Eleven men were bitten by irradiated infected mosquitoes. The other 41 men were bitten either by irradiated noninfected mosquitoes or by a few nonirradiated infected mosquitoes (see below). The number of mosquitoes per exposure varied from 7 to 500. Saline infusions were started in volunteers before each exposure to mosquitoes in case rapid intravenous administration of drugs was required to counteract allergic reactions to mosquito bites. Irradiated mosquitoes were allowed to feed on volunteers for about 20 min and each blood-engorged mosquito was then dissected to determine whether its salivary glands contained sporozoites. Control volunteers were bitten by a comparable number of noninfected irradiated mosquitoes. In addition, one or more volunteers were usually exposed to a small number of nonirradiated infected mosquitoes in order to verify the infectivity of irradiated mosquito batches. The immunization procedure was repeated with the same volunteers 2-8 times at intervals of 2 or more weeks.

Following completion of their immunization schedules, volunteers were challenged with nonirradiated infected mosquitoes approximately 2 weeks after their last exposure to irradiated mosquitoes. On each occasion, at least one control volunteer was also bitten by the same mosquitoes in order to verify transmission potential. Mosquitoes were divided into 2

groups and allowed to feed intermittently on immunized and control volunteers by the interrupted bite technique (4). After feeding, mosquitoes were dissected to determine the actual number that were infected.

The protective effects of immunization were determined by examination of thick blood films obtained from control and immunized volunteers. Blood films were obtained 7 days after challenge and, depending on the development of patent parasitaemia, daily for the next 3 weeks, every second day for the following 2 weeks, and twice a week for another 4½ months. Immunized volunteers who failed to develop parasitaemia within several weeks after challenge were exposed again to mosquitoes infected with a homologous or heterologous strain of *P. falciparum*. Volunteers who developed an acute clinical attack of malaria were given antimalarial treatment to cure their infections. As a precautionary measure, radical curative treatment was also administered to participants who did not develop patent parasitaemia during the study. Participants who were exposed to *P. vivax* sporozoites received the standard chloroquine-primaquine treatment and those exposed to *P. falciparum* sporozoites were cured with chloroquine (exposure to Tamenie strain) or amodiaquine-tetracycline (exposure to Marks strain).

RESULTS

Immunization against *P. vivax*

Three persons who were exposed to irradiated mosquitoes infected with *P. vivax* were not protected against subsequent challenge with the homologous strain. They were bitten by irradiated mosquitoes on 4 occasions at intervals of 2–4 weeks. The prepatent period, development of parasitaemia, and clinical course of infection in these volunteers were similar to those observed in control volunteers.

Immunization against *P. falciparum*

Exposure to fewer than 200 irradiated infected mosquitoes on 2–4 occasions. Four volunteers who were exposed on 2–4 occasions to irradiated mosquitoes infected with the Tamenie strain of *P. falciparum* were not protected against subsequent challenge with the homologous strain. They were each bitten by a total of fewer than 200 irradiated infected mosquitoes over a period of 1–4 months. During the course of immunization, 2 of the volunteers developed patent infections, which were cured immediately with chloroquine. The course of infection in these individuals after challenge was similar in every respect to that observed in control volunteers.

Exposure to 440 or more irradiated infected mosquitoes on 6–8 occasions. Three volunteers—L.A., D.S. and W.D.—were immunized over a period of 10–38 weeks by allowing a total of 440–987 irradiated mosquitoes infected with the Tamenie strain of *P. falciparum* to bite them on 6–8 occasions (Fig. 1).

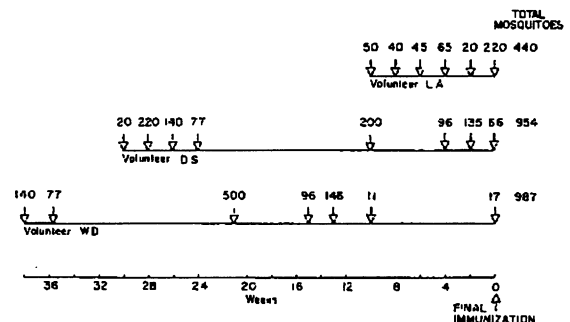


Fig. 1. Immunization schedule of 3 protected volunteers. Irradiated mosquitoes infected with the Tamenie strain of *Plasmodium falciparum* from Ethiopia were allowed to introduce the sporozoite antigen by feeding. Numbers above the arrows indicate the number of infected mosquitoes feeding on the volunteer.

Volunteer L.A. was exposed to irradiated mosquitoes on 6 different occasions and the interval between exposures was precisely 2 weeks on each occasion. Dissection of blood-fed mosquitoes over the 10-week period revealed that he had been bitten by a total of 440 mosquitoes infected with the sporozoites. Two weeks after the last immunizing exposure, L.A. was challenged with 13 nonirradiated mosquitoes infected with the same strain of *P. falciparum*. He did not become infected, whereas the control volunteer developed patent parasitaemia 12 days later. However, after being challenged again with 45 infected mosquitoes, 16 weeks after the last immunizing dose, L.A. developed an infection during the normal prepatent period (Table 1).

Volunteer D.S. was exposed to irradiated mosquitoes on 8 different occasions and was bitten by a total of 954 irradiated mosquitoes containing sporozoites. The first 4 immunizing exposures were given at regular intervals every 2 weeks. There was then an interval of about 13 weeks between the fourth and fifth exposures. A period of 6 weeks elapsed before the next exposure, and the last 2 exposures were given once more at intervals of 2 weeks. The course of immunization extended over a period of 30 weeks. Two weeks after the last immunizing dose, D.S. was bitten by 14 nonirradiated mosquitoes infected with the

Table 1. Challenge of immunized volunteers^a with sporozoites of two strains of *Plasmodium falciparum*

Weeks following final vaccination	Volunteer L A			Volunteer D S			Volunteer W D		
	Strain	No of mosquitos	Patency	Strain	No of mosquitos	Patency	Strain	No of mosquitos	Patency
2	Tamenie ^b	13	No	Tamenie	14	No			
4									
6									
8				Tamenie	12	No	Marks ^c	9	No
10									
12									
14									
16	Tamenie	45	Yes						
18				Marks	7	Yes	Tamenie	8	Yes

^a All control volunteers became positive within 14 days after being bitten by the same mosquitos used to challenge immunized volunteers

^b The Tamenie strain originated in Ethiopia

^c The Marks strain originated in Viet Nam

homologous Tamenie strain. He did not develop a patent infection. Approximately 8 weeks after the last immunizing dose, he was rechallenged by 12 mosquitos infected with the same homologous strain and, again, he was fully protected. On both occasions, control volunteers developed patent infections within 2 weeks after challenge. The immunized volunteer was not protected after challenge by 7 mosquitos infected with the heterologous Marks strain of *P. falciparum* 17 weeks after the last immunization (Table 1). He was also not protected against challenge with the homologous Tamenie strain 25 weeks after the last immunization.

Volunteer W.D. was exposed to irradiated mosquitos on 7 different occasions and was bitten at irregular intervals over a period of 38 weeks by a total of 987 irradiated mosquitos containing sporozoites. About 8 weeks after the last immunizing dose, he was challenged with 9 mosquitos infected with the Marks strain and was protected against this heterologous challenge, whereas 2 control volunteers became patent within 11–13 days after challenge. Eighteen weeks after his last immunizing dose, the immunized volunteer was challenged again by mosquitos infected with the homologous Tamenie strain, and developed patent parasitaemia 13 days later (Table 1).

DISCUSSION

The results of the investigations described in this report indicate that protection against malaria can be induced in man by inoculation of irradiated sporo-

zoites of *P. falciparum*. Considered together with findings reported earlier (5–8), 5 persons have been successfully vaccinated against falciparum malaria. Under our experimental conditions, protection was achieved only after 6, 7, or 8 exposures to a total of 440–987 irradiated infected mosquitos. Exposure to a total of fewer than 200 irradiated infected mosquitos on 2–4 occasions failed to protect 4 individuals against *P. falciparum* and 3 individuals against *P. vivax*. Individuals who were protected did not develop patent parasitaemia, whereas those who were not protected developed patent infections; these infections were indistinguishable from those observed in nonimmunized persons. The results agree with observations in animal models that immunity induced to sporozoites does not extend to the blood stages of the malaria parasite (9, 10).

Exposure to irradiated infected mosquitos could not be carried out at well-defined intervals or with a constant number of infected mosquitos since immunization depended on the availability of gametocyte donors to infect the mosquitos. Difficulty in obtaining gametocyte donors was due to variability in the response of infected individuals to suppressive drug regimens, in the duration and intensity of subsequent gametocyte levels, and in the infectivity of gametocytes to mosquitos. These difficulties, considered together with the problem of estimating the number of sporozoites released during mosquito feeds, preclude any definite estimates of sporozoite inocula or optimum immunization schedules at the present time.

During the course of immunization, patent parasitaemia developed in 2 of the 7 persons who were

bitten by fewer than 200 mosquitos. These observations confirm those made by Clyde et al. (6) that irradiation with 120 Gy is not always effective in rendering all sporozoites noninfective to man. Further studies are needed to determine the minimum amount of irradiation that is required to ensure that no sporozoites remain infective.

Protection was observed for at least 8 weeks following 6–8 exposures, but it was no longer evident 16–18 weeks after immunization. Of particular importance is the fact that this protection extended to a strain of *P. falciparum* geographically remote from the strain used during the course of immunization. These observations suggest that antigenic variation, observed among asexual erythrocytic stages (11), may not interfere significantly with the induction of immunity by sporozoites.

The results obtained in these studies are essentially

in agreement with those described by Clyde et al. (5, 6) and they provide substantial evidence that attenuated sporozoites can protect people against falciparum malaria. In view of current technological constraints, it seems unlikely that a sufficient number of sporozoites would be forthcoming to immunize large population groups. On the other hand, a sporozoite vaccine might be produced for administration to small groups of nonimmune individuals in areas of high malaria endemicity. With the availability of an effective vaccine, such individuals would no longer have to be concerned about taking prophylactic drugs at regular intervals or becoming infected with drug-resistant strains of *P. falciparum*. The successful immunization of human volunteers reported here, and by others, should encourage continuing efforts toward the development of a sporozoite vaccine against human malaria.

RÉSUMÉ

EMPLOI DE SPOROZOÏTES ATTÉNUÉS DE *PLASMODIUM FALCIPARUM* POUR L'IMMUNISATION DE VOLONTAIRES HUMAINS

Après leur immunisation au moyen de sporozoïtes atténués de la souche éthiopienne Taménie de *Plasmodium falciparum*, on a constaté chez trois volontaires humains une protection efficace contre les sporozoïtes normaux. Les sporozoïtes ayant opéré l'immunisation avaient été atténués en soumettant les moustiques porteurs (*Anopheles stephensi*) à une dose de rayonnements ionisants d'au moins 120 Gy (12 000 rad). Les moustiques infectés et irradiés ont pu ensuite se nourrir sur les volontaires et injecter ainsi dans leur sang des sporozoïtes. Pendant les 10 à 38 semaines qu'a pris ce processus d'immunisation et à intervalle de temps variable, des volontaires ont été exposés 6 à 8 fois aux piqûres de 440 à 987 moustiques infectés et irradiés au total. La protection contre une infection ultérieure par des moustiques non irradiés a duré au moins 8 semaines après la dernière "injection" de sporozoïtes irradiés mais elle avait pris

fin lors d'épreuves faites les 16ème ou 18ème semaines. Ceux des volontaires qui n'avaient été exposés qu'à un nombre de moustiques égal ou inférieur à 200 en 2 à 4 fois n'ont pas, en revanche, été immunisés contre l'infection par des moustiques non irradiés. L'immunisation par les piqûres d'un nombre suffisant de moustiques irradiés porteurs du parasite de la souche éthiopienne Taménie sensible à la chloroquine s'est révélée également capable de protéger contre la souche vietnamienne Marks de *P. falciparum* résistant à la chloroquine. Ces résultats donnent à penser que l'immunisation au moyen de sporozoïtes atténués peut constituer une méthode utile pour protéger de petits groupes d'individus non immuns vivant dans des zones d'endémie. Ils justifient la poursuite des efforts visant à mettre au point un vaccin à base de sporozoïtes contre le paludisme humain.

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